Further identification of the dye as thionol has not been attempted, nor have tests been made for the presence of the intermediate oxidation product, namely, phenothiazone.

The results obtained suggest that the oxidation of phenothiazine which normally takes place slowly in the presence of moisture, light, and air is greatly facilitated by the distribution of the phenothiazine over the extensive surface present in finely divided bentonite. That this is a fairly general phenomenon is shown by preliminary experiments in which dimethylacridan and diphenylamine have been oxidized to colored compounds by grinding with bentonite.

If compounds of low-water solubility are of insecticidal and fungicidal value by virtue of slow oxidation to a more potent compound, it may well be that a mixture of these compounds with bentonite will improve their efficiency.

Recently, M. C. Goldsworthy and E. L. Green of the Bureau of Plant Industry, U. S. Department of Agriculture, claimed to have discovered that the fungicidal action of phenothiazine is due to its partial oxidation to phenothiazone, according to the following statement quoted from U. S. Department of Agriculture press release dated February 11, 1940:

"An attempt has been made to find a way to spray the chemical so that some of it would change to phenothiazone and kill fungi and the remainder keep its original form as an insect killer. Some promising results were obtained by mixing phenothiazine with bentonite—a fine clay—and hydrated lime. This caused the spray to stick to the fruit and build up a residue. The under part of the spray film apparently retains its insecticidal value while that exposed to light on the surface of the film changed to a fungicide."

The experiments reported here suggest that the distribution of phenothiazine over the extensive surface presented by bentonite permits intimate contact with atmospheric oxygen, thereby facilitating oxidation, an action which may be further aided by the alkalinity of the hydrated lime.

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Electrophoretic Analysis of Digested Antitoxic Sera.

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Recent work has shown that certain antitoxins can be partially digested[1] with pepsin without losing their abilities to neutralize

toxin. The chemical changes[2] that accompany this alteration are apparently small; the immunological modification[3] is, however, clearly set forth in the seriously impaired antigenicity of digested antitoxins. We have made electrophoretic studies of a number of such digested antitoxic sera. In this way it has been possible to follow the course of the enzymatic digestion and to obtain an objective record of the changes that occur when sera are modified.

In these experiments diphtheria and tetanus antitoxic horse sera have been digested with pepsin at 37°C and pH 4. Electrophoretic analyses have been made of the original sera and of samples of the digest mixture taken at various intervals during the digestion process.

Previous work has shown that powerfully antitoxic horse sera contain large new T-components[4] which develop during the course of hyperimmunization. Such a T-peak appears in the electrophoretic pattern of the diphtheria and tetanus antitoxic sera shown

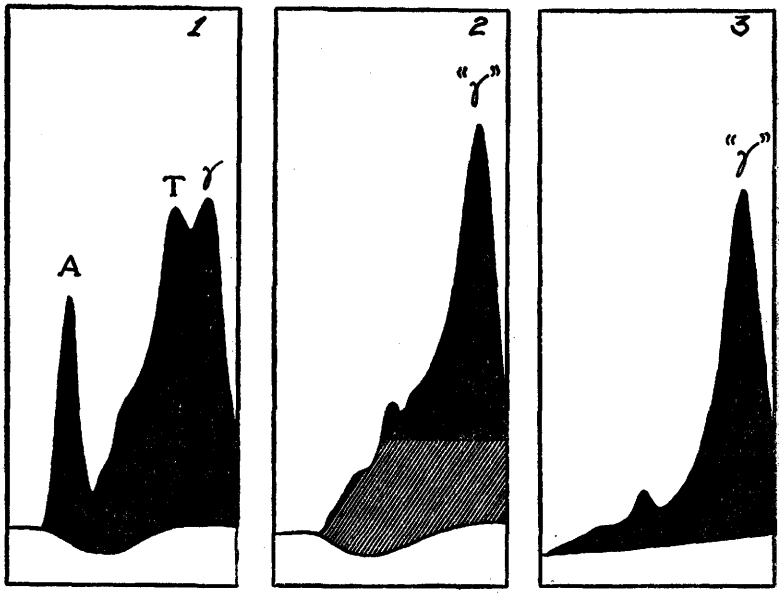

FIG. 1. Electrophoretic diagram of a defibrinated diphtheria antitoxic horse plasma (No. 4635) diluted 1:4 and dialyzed against pH 7.6 buffer-saline.

FIG. 2. Electrophoretic diagram of plasma No. 4635 after one-half hour's digestion.

FIG. 3. Diagram of plasma No. 4635 after digestion for 100 hours.

1 Parfentjev, I. A., U. S. Patents No. 2065196 and No. 2123198.

2 Pope, C. G., *Brit. J. Exp. Path.*, 1938, **19**, 245; 1939, **20**, 132, 201.

3 Weil, A. J., Parfentjev, I. A., and Bowman, K. L., *J. Immunology*, 1938, **35**, 399.

4 van der Scheer, J., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 427; van der Scheer, J., Wyckoff, R. W. G., and Clarke, F. H., *J. Immunol.*, 1940, **39**, 65.

in Figs. 1 and 4. A study of areas and mobilities in the electrophoretic diagrams demonstrates that during digestion this T-component is more or less rapidly converted into a slower component that moves at substantially the same rate as the normal γ-globulin. As can be seen from Fig. 2 no T is left in a diphtheria antitoxin after one half hour's digestion. A great advantage of digested antitoxin lies in the comparatively slight reactions that follow its injection into man; in order to modify it to this extent, however, a serum must be digested for many hours. Loss of reactivity is not therefore the immediate consequence of the transformation of the T-globulin. A comparison of Fig. 2 and 3 shows that prolonged digestion does not further alter the mobility of the transformed T component but it does eliminate electrophoretically-heterogeneous material such as that which has been shaded in Fig. 2. It is not improbable that reactivity on injection is associated with this heterogeneous protein.

Steps in the conversion of the T-globulin can be seen in Figs. 4-6. After only 10 minutes' digestion (Fig. 5) some, but not all, the T has disappeared; at the end of 30 minutes it has been completely transformed (Fig. 6).

In a later publication we shall discuss in greater detail the electrophoretic evidence concerning the action of pepsin on immune sera and shall compare this evidence with measurements of the loss of coagulable protein and of antibody activity during digestion.

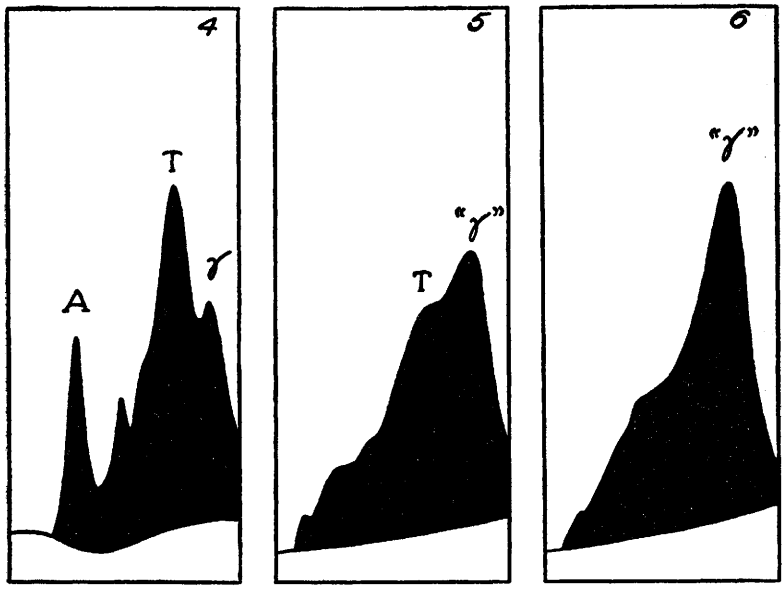

FIG. 4. Diagram of a tetanus antitoxic horse serum No. A787.
FIG. 5. Diagram of serum No. A787 after 10 minutes' digestion.
FIG. 6. Diagram of serum No. A787 after one-half hour's digestion.